

MORPHOLOGY OF THE GENUS CEPHALOSPORIUM, WITH DESCRIPTION OF A NEW SPECIES AND A VARIETY

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(WITH PLATES 50 AND 51, CONTAINING 9 FIGURES)

Certain species of the hyphomycetous genus *Cephalosporium* have been found to be of common occurrence in the humus-rich, prairie soils of Iowa. The morphology and relationships of this genus have not been well understood; it is therefore believed of interest to publish the results of a study of one of these species and a variety.

The genus *Cephalosporium* Corda is characterized by its well-developed hyaline mycelium and its slender, unbranched conidiophores which abstrict non-septate spores from the tip, these latter being pushed to one side by the later spores and all remaining as a head, stuck together by mucus. The genus *Hyalopus* Corda is differentiated from *Cephalosporium* by the abundant production of mucus, and the resultant globular refractive head produced. The distinction is made solely on the relative amount of mucus. *Allantospora* Wakk has been separated from *Hyalopus* on account of its allantoid, sometimes 1-2 septate, conidia. One species of *Allantospora* has been described, *A. radiculicola* Wakk, upon the roots of *Saccharum officinarum*. Its obvious relationship to *Cephalosporium* has caused Saccardo (Sylloge 14: 1043) to include it among the Amerosporae of the Mucedinaceae, while Clements in his "Genera of Fungi" has placed it under the Phragmosporae (*Hyalophragmiae*), doubtless because of the occurrence of septate spores. Lindau (Rabenhorst's Krypt. Flora 8: 100-101) has expressed the opinion that the genera *Hyalopus* and *Cephalosporium* should be united. Under the heading of "*Hyalopus populi* Nypels" he says: "Bei kulturen in feuchten Luft entsteht

das durch Schleim zusammengeballte kuglige Konidienköpfchen. Dagegen zeigen die Konidienträger in trockner Luft nur einzelne Konidien welcher locker zu Köpfchen zusammentreten. In letzteren Falle zeigt sich gegen *Cephalosporium* nicht der geringste Unterschied, so dass ich der Meinung sei möchte, *Hyalopus* ist nur ein unter feuchten Verhältnissen wachsendes *Cephalosporium*. Weitere Beobachtungen über die Bildung der Konidienköpfchen wären sehr erwünscht."

The species and variety of *Cephalosporium* here described were isolated from humus-rich soil on dextrose agar made up without intentional addition of combined nitrogen in any form. The colonies develop quickly on a variety of media, peptone agar and gelatin, mannite and dextrose N free agar, mannite dextrose and starch N free solutions, peptone solution, peptone and dextrose gelatin and solidified blood serum. The rapidity, type and luxuriance of growth exhibit some differences on the various media employed.

The mycelium is in all cases hyaline, at least when young, septate and much branched. The sterile hyphae are of indeterminate length. On dextrose agar plate cultures they grow from center to circumference of the Petri dish in the course of four days to a week at 20° C. The diameter of the hyphae varies from 5 to 25 μ . The cell contents, at first homogeneous, become somewhat vacuolate and later hold a large number of oil drops. There is little evidence of negative chemotropism of the hyphae toward each other, as they cross and recross repeatedly. The hyphae penetrate the medium to a depth of half an inch in agar tubes. The organism grows well only in the presence of an abundance of oxygen. Aerial hyphae are not thrown off in abundance from the surface of a plate culture, but whenever the organism comes in contact with a foreign substance as the glass wall they are sent up in abundance. Some of these may reach a length of half an inch or more.

The conidiophores are developed abundantly on all hyphae that lie at the surface of the medium and upon the aerial hyphae when formed. A few develop even below the surface of the medium. They are slender, hyaline, and vary in length from a micron or two to twenty or thirty on some aerial hyphae and 10 to 50 μ on a moist surface or in a moist atmosphere. They are usually non-

septate. They show little evidence of the negative hydrotropism so characteristic of many of our common molds, such as *Penicillium* and *Aspergillus*. Those developing from hyphae lying in contact with the medium are usually bound down by the water film and develop along its surface.

The spores are formed by the abstriction of the tip of the sporophore. Each is enveloped in mucus, the amount depending upon the moisture of the atmosphere in which it develops. In a dry atmosphere only sufficient is found to cause the spores to stick together in a head. In a moist atmosphere the globule of mucus swells until it completely envelops the spores, and careful observation will show them floating free in the liquid, which sometimes has three to four times the mass of the spores. That this is not merely water but mucus, is demonstrated by the preparation of a mount in alcohol, where the heads remain intact, because the mucus does not dissolve. When water is added, however, the spores are freed by the solution of the mucus and separate. Lindau's characterization of *Hyalopus* as a *Cephalosporium* grown in a moist atmosphere is justified. The heads vary in size from 10 to 35 μ and contain from two to numerous spores. The spores are ovoid to cylindric with rounded ends, usually with granules. When the sporophores are short, the spore masses are found upon the surface of the hyphae. The sporophore sometimes produces a head of spores, then, because of some undetermined stimulus, it resumes growth and produces a new head. This may occur several times and results in masses of spores at intervals along the sporophores. The spores developed on the moist surface of the medium are usually larger than those of the aerial conidiophores. When formed they frequently continue to enlarge after separation from the hyphae and become considerably elongated, even crescent-shaped, and falcate. When grown to several times their original length they become septate, from one to six or eight septa being formed. These spores then bud at one or more points and develop new conidia of a similar size and shape. In this manner large masses of sickle-shaped or allantoid, septate conidia are produced. They remain attached to each other by slender threads. Many of these spore masses in the older portion of the culture are distinctly visible to the naked eye. Rarely they reach a diameter of several millimeters. In some cases the spore mass has a

greenish tinge. In an atmosphere sufficiently moist some of the erect conidiophores are found to be capped by these long septate spores rather than by the more usual short, non-septate type. Every gradation in shape, size and septation may be observed in a single mount from some cultures. These latter allantoid, septate spores imbedded in mucus answer to the diagnosis of *Allantospora*. The spores of all types germinate readily. Germ tubes may issue from several of the cells of a septate spore. The spores borne on aerial conidiophores and forming heads of the *Cephalosporium* type are from $4-15\mu$ in length and one half to one third as broad. Those that develop in a moist atmosphere and form heads of the *Hyalopus* type vary from 5 to 15μ and are one fourth to one half as broad as long. When developed on the surface of the medium in the presence of an excess of moisture, they either resemble the preceding or become allantoid or falcate, $20-30 \times 3-5\mu$.

From the foregoing description it seems evident that the genus *Hyalopus* should be merged with *Cephalosporium* as suggested by Lindau. It is also possible that *Allantospora* is but a growth form of *Cephalosporium*.

The variety *purpurascens* described below differs in no marked particular from the type except for the production of a purple pigment. This pigment production is somewhat inconstant, and is usually slow in making its appearance. In some cases the purple coloring matter is diffused into the dextrose agar in such quantities as to make it practically opaque and of a rich wine-red color. The pigment permeates the mycelium and is found in some of the spores.

It is possible that the following species has been observed and described in one of its growth forms, but none of the published diagnoses are sufficiently complete to allow of satisfactory identification.

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***Cephalosporium Pammellii* sp. nov.**

Hyphis sterilibus decumbentibus vel raris in aere crescentibus, longis, hyalinis, multis ramosis, vacuolatis denique protoplasmate cum oleis globulis repleto, articulatis, $5-15\mu$ crassis; chlamydo-

sporis $5-10 \times 10-15 \mu$ conidiophoris e mycelio lateraliter nascentibus, erectis vel decumbentibus, simplicibus vel raris ramosis, non vel raris septatis, $2-20 \times 3-5 \mu$ in aere sicco, $5-50 \times 3-8 \mu$ in aere humido, non hydrotropismis, summo capitulo globoso $10-35 \mu$ diam.; conidiis in aere humido muco-glomeratis, in aere sicco separatim vel laxe cohaerentibus, ellipsoideis vel ovoideis et continuis, vel in aqua falcatis, allantoideis et $1-8$ septatis, hyalinis, granulosis.

Hab. in dextrose agar pulvene terrae humosae infecta.

Var. *purpurascens*. Hyphis sterilibus primo hyalinis, albis, denique roseis vel purpureis; conidiis hyalini vel purpurascens, pigmento purpureo in alcohol et aqua soluto, in dextrose-agar et alteris mediis saccharis diffuso.

Hab. in dextrose agar pulvere terrae humosae infecta.

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EXPLANATION OF PLATE 50

Cephalosporium Pammelii Buchanan

1. Microphotograph of type. Mycelium, sporophores and spores on the surface of dextrose agar plate. Courtesy of Iowa Agricultural Experiment Station.
2. Microphotograph of type. Masses of falcate and allantoid, septate spores. Surface of dextrose agar plate. Courtesy of Iowa Agricultural Experiment Station.

EXPLANATION OF PLATE 51

Cephalosporium Pammelii and var. *purpurascens*

1. Aerial conidiophores developing in relatively dry air. 1a. Medium moist air, therefore somewhat moist, mucus globule not evident. 1b. var. *purpurascens*. Conidiophores in dry air after medium has dried. 1c. var. *purpurascens*.
2. Conidiophores which have grown in length intermittently, with clusters of spores. 2a. var. *purpurascens* developed in relatively dry air. 2b. Type developed in moist air with mucus globules.
3. Aerial conidiophores of type, developed in moist air, showing the *Hyalopus* type of head.
4. Conidiophores of type produced in contact with the surface of moist agar. 4a, b, c, d. Illustrating the variety of spore shapes and sizes, and the evident continued growth of the spore after abstraction.
5. Falcate spores from a large spore mass, illustrating the method of budding and continued growth.
6. Spores from the surface of agar, showing the unicellular and multiseptate forms. 6a. Septate spore from preparation stained with fuchsin to show the nuclei. 6b. Septate spores of var. *purpurascens* with purple pigment.
7. Chlamydospores of the type, from dextrose N free agar. 7a, b, c, d, e, showing development of spores, intercalary or terminal.